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Published in:
Journal of Agricultural and Food Chemistry

DOI:
10.1021/acs.jafc.7b02604

Publication date:
2017

Document version
Peer reviewed version

LAYERED DOUBLE HYDROXIDES: POTENTIAL RELEASE-ON-DEMAND FERTILIZERS FOR PLANT ZINC NUTRITION

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ABSTRACT

A novel zinc (Zn) fertilizer concept based on Zn doped layered double hydroxides (Zn-doped Mg-Fe-LDHs) has been investigated. Zn-doped Mg-Fe-LDHs were synthetized, their chemical composition was analyzed and their nutrient release was studied in buffered solutions with different pH values. Uptake of Zn by barley (*Hordeum vulgare* cv. Antonia) was evaluated in short- (8 weeks), medium- (11 weeks) and long-term (28 weeks) experiments in quartz sand and in a calcareous soil enriched with Zn-doped Mg-Fe-LDHs. The Zn release rate of the Zn-doped Mg-Fe-LDHs was described by a first-order kinetics equation showing maximum release at pH 5.2, reaching approximately 45% of the total Zn content. The Zn concentrations in the plants receiving the LDHs were between 2- and 9.5-fold higher than those in plants without Zn addition. A positive effect of the LDHs was also found in soil. This work documents the long-term Zn release capacity of LDHs complying with a release-on-demand behavior and serves as proof-of-concept that Zn-doped Mg-Fe-LDHs can be used as Zn fertilizers.

Keywords

Fertilizer, layered double hydroxide, nanomaterial, plant, Zn.

1. INTRODUCTION

Zinc (Zn) is an essential element required for the normal growth and development of all living organisms, including humans and plants.[1] Zinc stimulates the activity of more than 300 enzymes in plants and is required for the maintenance of membrane integrity, protein synthesis, and carbohydrate and auxin metabolism, among other functions.[2] Although the global risk of human Zn deficiency has decreased in the last 25 years, 1.1 billion people, of which approximately 90% live in Africa and Asia, were still at risk of Zn deficiency in 2011 because of an inadequate dietary supply.[3] In crops, Zn deficiency causes yield reductions, stunted growth, widespread infestation by various diseases and pests, and lower fertilizer use efficiency.[4] The availability of Zn in soil to plants is influenced by multiple factors such as the pH; redox conditions; and contents of organic matter, carbonate and phosphate.[5]

The application of Zn-containing fertilizers represents a rapid and effective approach to improve grain Zn and increase crop production.[6] Zinc sulfate is the most commonly used Zn fertilizer. Other Zn compounds used include chloride, nitrate, and Zn oxide.[5,7] However, when these fertilizers are applied to Zn-deficient calcareous soils, only a small proportion becomes available to plants [8] because the high pH in these soils increases the binding of Zn in hydroxides and oxides as well as the adsorption of Zn to soil iron and aluminum oxides, clays and calcite.[9] In acid soils, plant Zn deficiencies have been associated with high Zn mobilization and subsequent losses by leaching. Chelation with EDTA (ethylene diamine tetraacetic acid) or similar compounds is one of the strategies used to increase the availability of Zn.[4,10] However, these chelating agents also bind other metal ions such as Ca^{2+}, Fe^{3+}, Al^{3+} or Cu^{2+} present in soils, which may reduce the stability of the Zn chelates.[11] Natural organic Zn chelates and lignosulfonates have also been studied and appear to have potential for use in weakly

acidic soils, but their efficiency appears to be limited under conditions of high soil pH.[10,12,13] Further work is therefore required to develop efficient Zn fertilizers that can sustain crop production and crop quality in Zn deficient soils.

The term 'enhanced efficiency fertilizers' (EEFs) has been adopted.[14] While the current EEFs are either slow-release, following microbial degradation of their polymeric coating,[15] or controlled-release via slower spontaneous dissolution, [16] others could be based on release-on-demand by actively growing plants. A first step towards formulating such fertilizers involved the synthesis of an organomineral matrix composed of metal [Mg, Zn, Fe]-humic phosphates. [17] This product was described as a "rhizosphere-controlled fertilizer" (RCF) containing two main nutrient fractions: a water-soluble fraction, or "starter" fraction, and a "rhizosphere-controlled" fraction, which is insoluble in water but solubilized by the action of rhizospheric acids (e.g., citric and malic acids) and the protons released by plants and microorganisms. RCF-based P fertilizers were able to facilitate the recovery of P-stressed plants, indicating the suitability of the RCF strategy for the preparation of mineral fertilizers with a nutrient release pattern sensitive to plant nutritional needs.[18]

Layered double hydroxides (LDH) are 2-D nanostructured synthetic materials with ion-exchange properties and potential demonstrated in technological applications such as drug delivery systems,[19–21] ion exchangers/sorbents,[22] and polymer reinforcement and catalyst support.[23] LDHs consist of alternating layers of positively charged metal hydroxides and interlayers of charged compensating anions (Figure 1). The metal hydroxide layers typically include both a divalent and a trivalent metal cation, which occupy the center of an octahedron with hydroxyl groups located at the edges. The cations are typically Mg^{2+} and Fe^{3+}/Al^{3+}, but others can also be substituted into the metal hydroxide layers.[24] In addition, many different interlayer anions can be accommodated, e.g., carbonate, nitrate,

sulfate, phosphate, selenate and molybdate, as well as many organic anions including surfactants. To date, the application of LDH compounds in agriculture has been based on their ion exchange properties, with anions hosted in the interlayer. LDH compounds have been used as controlled-release hosts for agrochemicals,[25] including anionic nutrients such as nitrate[26–29] and phosphate and organic compounds such as chelating agents, pesticides and herbicides[30–32] accommodated within the interlayers. LDHs have a high affinity for bicarbonate/carbonate anions and will favor the incorporation of these anions when the alkalinity in the rhizosphere increases. Hence, bicarbonate/carbonate may exchange with interlayer nitrate and phosphate.[33] The results of all the studies conducted to date indicate the same mechanism of action: anions are slowly released/adsorbed in the interlayer similarly to what occurs on the surface of clays.

In addition to interlayered anions, a pH-controlled release of layered cations (Mg^{2+} and Zn^{2+}) from a pyroaurite-type LDH (Mg-Fe-LDH) doped with Zn has recently been reported in short-term (<250 min) laboratory experiments carried out under controlled conditions in the absence of carbon[34]. The LDHs were stable at pH > 7 but dissolved slowly at lower pH, releasing their constituent cations into the media. Although both Mg and Zn can be released, the potential of LDHs as Zn fertilizers appears to be more favorable since the quantities needed by plants are much lower (approximately 100-1000 times less than for Mg), thereby reducing the application costs.

According to the dissolution of Zn at acidic pH values, a controlled release of the nutrients accommodated in the metal hydroxide layer of LDHs is expected as a consequence of the decreasing pH of the rhizosphere following root excretion of protons, low molecular weight organic acids (OAHs), and/or CO_2.[35]

In this work, the potential of Zn-doped Mg-Fe-LDHs as Zn fertilizers was examined. The experimental work tested the following hypotheses based on the known characteristics outlined above: 1) Zn-doped Mg-Fe-LDHs will be stable in water, and fully/partially dissolved by acids; 2) the kinetics of Zn dissolution will be in synchrony with plant uptake in pot experiments; and 3) the combined application of Zn-doped Mg-Fe-LDHs with ammonium will increase plant Zn uptake due to rhizosphere acidification enhancing the LDH solubility.

2. MATERIALS AND METHODS

2.1.LDH synthesis and chemical analysis

Two Zn-doped Mg(II)-Fe(III) LDHs (Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3) were prepared, and their chemical structures were verified by powder X-ray diffraction (pXRD) and FT-IR spectroscopy (see Supporting Information). Briefly, Zn-doped Mg-Fe-LDH-NO_3 was prepared using a constant pH co-precipitation method involving the drop-wise addition of 100 mL of an aqueous solution of 0.1 M $Fe(NO_3)_3.9H_2O$ (PA, Sigma Aldrich) to 100 mL of a mixed solution of 0.2 M $Mg(NO_3)_2.6H_2O$ (PA, Sigma Aldrich) and 0.0158 M $Zn(NO_3)_2$ (PA, Sigma Aldrich) [molar ratio, Zn/(Zn+Mg+Fe) = 0.05] at a constant pH of 9.5 ± 0.2 with the simultaneous addition of carbonate-free 1.0 M NaOH to the reaction mixture over 2 h with continuous stirring. To avoid the carbonate contamination, all solutions were bubbled with Ar. The Zn-doped Mg-Fe-LDH-CO_3 form was obtained by dispersing the nitrate form in a 0.1 M Na_2CO_3 solution for 3 h to exchange the interlayered nitrate with carbonate. The suspensions were dried in an oven for 24 h at 40°C and milled in an agate mortar to obtain fine powder materials.

111 The synthesized Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3 were analyzed according
112 to the standard methods described in the European Regulation for Micronutrient Fertilizers (EC
113 2003/2003),[36] which include the analysis of the total (HCl 35%), water-soluble, citric acid-soluble
114 (2%), and DTPA-TEA-soluble (diethylene triamine pentaacetic acid-triethanolamine) fractions[37] of Zn,
115 Fe, and Mg. The fraction soluble in citric acid simulates the fraction of fertilizer insoluble in water but
116 potentially solubilizable by rhizospheric acids, [17] whereas the DTPA-TEA-soluble fraction corresponds
117 to the fraction of metals available for plants. [37] The extracts were acidified when needed, and the total
118 Zn, Fe, and Mg contents were analyzed by flame atomic absorption spectrophotometry (AAS, Perkin
119 Elmer AA-3300).

120 **2.2.Nutrient release in aqueous solution as a function of pH and time**

121 Five milligrams of LDHs were incubated in 50 mL of a pH-buffered solution [10 mM 2-(N-
122 morpholino)ethanesulfonic acid (MES) or (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
123 (HEPES)] at different pH values (5.2 (MES), 5.5 (MES), 6.0 (MES), 7.1 (HEPES), 8.1 (HEPES)) in
124 closed plastic containers for 1, 3, 7, 14, and 28 days. The samples were gently agitated using an orbital
125 shaker at 20 °C and 50 rpm for 1, 3, 7, 14, and 28 days. Two replicates per combination of LDH,
126 blanks, pH and time were analyzed, giving a total number of 150 samples. After the specified amount
127 of time, samples were filtered through passed through 0.45 μm acetate filters, their pH was measured,
128 and the filtrate was finally diluted and acidified with 0.5 M HCl for the determination of total Zn, Fe,
129 and Mg by AAS. The remaining solid on the filters were recovered, dissolved in concentrated HCl (6
130 M) and similarly analyzed to check for the presence of undissolved or precipitated Zn, Fe or Mg. The
131 release data were fitted to the following first-order equation:

$$[M^{2+}]_t = a \cdot (1-e^{-b \cdot t}) \quad \text{Eq. (1)}$$

This was the best equation model based on the regression coefficients, as determined using SigmaPlot 13.0 software (Systat Software, Inc., San Jose, CA). $[M^{2+}]_t$ is the concentration of M^{2+} released (Zn^{2+} or Mg^{2+}, in mg M^{2+} per g of LDH) at time t (in days), "a" is the maximum concentration of M^{2+} that can be released, and "b" is the kinetic constant.

2.3.Plant experiments

The effectiveness of the Zn-doped Mg-Fe-LDHs in providing Zn to plants was assessed in four experiments in a greenhouse with minimum day/night temperatures of 18°C/15°C and a 16-h-day/8-h-night light regime. The first three were conducted using sand as the growth medium, whereas a calcareous soil was used in the fourth. The same experimental approach was used in the three experiments with sand as growth medium while varying the pot size and harvest time. Barley (*Hordeum vulgare* cv. Antonia) was grown in quartz sand media (Dansand® type 1 0.40-0.90 mm, 1.605 kg/m^3, 99.4 SiO_2%, pH 8.0) supplemented with the Zn-doped Mg-Fe-LDHs. The Zn-doped Mg-Fe-LDH dose was 10 mg per pot. Non-treated control plants were grown without any LDH or addition of other Zn source to the sand. The Zn media was prepared by mixing a portion of sand (22 g in the short-term experiment, 80 g in the medium term-experiment, and 70 g in the long-term experiment) with the LDHs in a closed plastic bottle by manual shaking for 30 min. For the short-duration experiments, 50 ml Falcon® polypropylene cone tubes with a 2 cm-diameter hole at the bottom were used as pots. The tubes were first filled with 18 g of sand, followed by 22 g of a sand-LDH mixture and finally 30 g of sand (70 g total). For the medium-duration experiments, 160 ml polypropylene cylindrical vessels with three 2 cm-diameter holes at the bottom were used. The vessels were first filled

153 with 60 g of sand, followed by 80 g of a sand-LDH mixture and finally 125 g of sand (265 g in total).
154 For the long-duration experiments, 1.1 L polyethylene cylinder pots with 90 mm external and 84 mm
155 internal diameters, 25 cm length and three 3 mm holes at the bottom were used as pots. The pots were
156 first filled with 330 g of sand, followed by 70 g of a sand-LDH mixture as a 1 cm layer and and finally
157 1250 g of sand (1650 g in total). The pots were protected from light exposure by covering with a dark
158 plastic film. The main experimental characteristics are presented in Fig. 2.

159 Barley seeds were humidified in the dark for 24 h using Milli-Q® type I ultrapure water with
160 continuous aeration. Then, 2 seeds were placed in each pot inside a small hole in the sand at the top.
161 The pot surface was covered with plastic film to preserve the humidity during the germination period.
162 After 14 days, seedling development was observed, and only one seedling was retained. Pot humidity
163 was controlled by weighing the pots and filling with either water or nutrient solution to achieve 80%
164 field capacity. Milli-Q® type I water was added every day to maintain the humidity in the pots, as
165 monitored by weighing, during the first two weeks. Next, nutrient solution was provided with all
166 nutrients except Zn and Fe. In the short- and medium-duration experiments, concentrations in the initial
167 nutrient solution applied were 0.2 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.3 mM $MgSO_4$ $7H_2O$, 0.1 mM NaCl,
168 0.3 mM $Mg(NO_3)_2$ $6H_2O$, 0.9 mM $Ca(NO_3)_2$ $4H_2O$, 0.6 mM KNO_3, 54 µM Na_2EDTA, 1.0 µM $MnCl_2$
169 $4H_2O$, 0.8 µM Na_2MoO_4 $2H_2O$, 0.8 µM $CuSO_4$ $5H_2O$, 2.0 H_3BO_3 and 1.0 $NiSO_4$ $6H_2O$. Then, the
170 concentrations were increased over the course of the experiment according to the growth rate (25 mg
171 dry weight (DW)/day) and water consumption of the plants (5-10 mL/day) (see Supporting Information
172 for concentration expressed per unit weight of substrate and the total nutrient quantities applied). In the
173 long-duration experiment, the same concentrations in the nutrient solution were used during the whole
174 experiment: 0.1 mM KH_2PO_4, 0.1 mM K_2SO_4, 0.15 mM $MgSO_4$ $7H_2O$, 0.05 mM NaCl, 0.075 mM

175 $Mg(NO_3)_2$ $6H_2O$, 0.225 mM $Ca(NO_3)_2$ $4H_2O$, 0.15 mM KNO_3, 27 µM Na_2EDTA, 0.5 µM $MnCl_2$
176 $4H_2O$, 0.4 µM Na_2MoO_4 $2H_2O$, 0.4 µM $CuSO_4$ $5H_2O$, 1.0 µM H_3BO_3 and 0.5 µM $NiSO_4$ $6H_2O$. The
177 total volume applied per plant over the course of the experiment was 2.04 L (see Supporting
178 Information for concentration expressed per unit weight of substrate and the total nutrient quantities
179 applied). When necessary, extra Type I water was added to achieve the correct pot weight.

180 Plants were grown in the pots for 8, 11 and 28 weeks for the short-, medium- and long-duration
181 experiments, respectively. Upon completion of the experiments, shoots were harvested, sequentially
182 washed with deionized water containing a few drops of detergent (Tween 20, Sigma Aldrich),
183 deionized water and twice in Milli-Q water (Millipore, Billerica, MA, USA), weighed to determine the
184 fresh weight; freeze-dried for 72 h, and weighed to determine the DW. The pots were carefully
185 removed, and the roots were recovered, washed and processed as described for the shoots.

186 Next, the samples were milled using a cyclone mill (President, Holbæk, Denmark) for digestion and
187 analysis. Samples were digested in a micro-scale microwave (UltraWAVE system, Milestone Srl,
188 Sorisole, Italy), as described elsewhere.[38] Multi-elemental analysis of the digested samples was
189 performed using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 5300
190 DV, PerkinElmer, Waltham, MA, US).

191 The fourth plant experiment was conducted in 1.8 L cylinder pots (28 cm diameter and 9 cm height)
192 filled with 1.125 Kg of a sandy soil (Kristianstad, Sweden; pH_{H2O} (1:5) 7.29, DTPA extractable
193 [37]micronutrients: 2.57 mg Zn/L, 18.1 mg Fe/L, 0.20 mg Mn/L mixed with 40% v/v coarse Leca®
194 (Light Expanded Clay Aggregate), 20% v/v perlite, and 0.1% (m/m) $CaCO_3$ in order to create porous
195 and alkaline soil conditions (pH 7.5). The Zn treatments included the two Zn-doped Mg-Fe-LDHs, a

positive control with Zn-EDTA and a deficient control where no Zn was added. The Zn dose applied was 1.5 mg Zn/pot (as Zn-doped Mg-Fe-LDH-NO_3, Zn-doped Mg-Fe-LDH-CO_3, or ZnEDTA). The Zn compounds were placed in the pots together with 100 mg N in calcium nitrate pellets (16% N) as described in Figure 2. Other major nutrients were applied by daily irrigation with a nutrient solution containing 800 µM $CaSO_4 \cdot 2H_2O$, 100 µM $Ca(H_2PO_4)_2 \cdot H_2O$, 300 µM $MgSO_4$, 600 µM K_2SO_4 and 100 µM NaCl. The total volume of nutrient solution used over the experimental period was 180 ml. Barley seeds were germinated in perlite for 5 days whereupon three vigorously germinating seeds were planted in the pots filled with moist (11% humidity) soil mixture containing the treatments. The plants grew for 7 weeks in a climate chamber with 16h day/8h night cycle at 23/19˚C and 60%/40% humidity. Two plants were harvested after 3 weeks while the remaining plant was grown for 7 weeks. Shoots were harvested and processed as described in the previous experiments. Once completion of the experiment, roots were removed and the remaining soil was collected and analyzed for pH and the Zn available fraction.[37]

3. RESULTS

3.1.Chemical analysis of the Zn-doped Mg-Fe-LDHs

The LDH concentrations of Zn, Fe, and Mg in the HCl-, citric acid-, DTPA-TEA- and water-soluble fractions are listed in Table 1. Derived from the total elemental concentrations (in HCl), the formula of the Zn-doped Mg-Fe-LDHs corresponds to $Mg_{0.613}Zn_{0.044}Fe_{0.341}(OH)_2 \cdot (NO_3)_{0.34} \cdot nH_2O$ (for Zn-doped Mg-Fe-LDH-NO_3) and $Mg_{0.613}Zn_{0.040}Fe_{0.340}(OH)_2(CO_3)_{0.178} \cdot nH_2O$ (for Zn-doped Mg-Fe-LDH-CO_3) (see Supporting Information for details of the formula determination). The ratio of divalent to trivalent metal cations in the solids was close to that of the starting solution. For both Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3, similar proportions of the elements were found after

hydrochloric and citric acid extraction. The proportion extractable by DTPA-TEA was lower than that extractable by the acids. The extraction with water did not dissolve any of the metals (Zn or Fe).

3.2.Nutrient release in aqueous solution

The total concentrations of Zn^{2+} and Mg^{2+} in solution of the incubated LDHs are shown in Figure 3 and Figure 4. For both cations, Mg^{2+} and Zn^{2+}, the maximum cation release as well as the rates clearly decreased as the pH increased (Table 2). The maximum Zn^{2+} dissolved was obtained at the lowest pH, 5.2, amounting to 46% and 41% of the total Zn in the nitrate and the carbonate forms of the LDHs (Table 1), respectively. Similar Zn^{2+} dissolution rates were obtained for both LDH forms, decreasing from 0.55 day^{-1} at the most acidic pH to 0.25-0.13 day^{-1} at the most alkaline pH. The maximum amount of Mg^{2+} dissolved was higher than that for Zn^{2+}. Thus, 74% of the Mg in the nitrate form and 62% in the carbonate form were dissolved at pH 5.2 (Table 2). Although a lower fraction of Mg^{2+} was released from the carbonate form, the rate was higher than for the nitrate LDH, as indicated by the higher "b" kinetic constants (Eq. 1). Low amounts of Zn^{2+} (less than 0.25%) were released at pH 8.1, while 38% and 27% Mg^{2+} were released from the nitrate and carbonate LDH forms, respectively, at the same pH. The presence of Mg^{2+} and Zn^{2+} in the residues remaining on the filters after filtration of the aqueous solutions suggests that these residues could be precipitates and/or undissolved or partially dissolved LDH.

3.3.Effect of the Zn-doped Mg-Fe-LDHs applied to sand growth media

3.3.1. Plant uptake of Zn

Three plant experiments were conducted in the sand growth media amended with the same Zn-doped Mg-Fe-LDH dose (10 mg of LDH/plant) to study the effect of these compounds over time.

In the three experiments, a clear positive effect of the Zn-doped Mg-Fe-LDHs on the Zn concentration (Table 3) and Zn accumulation (Figure 5) in plants was observed compared to the non-treated control plants, which contained very low Zn concentrations, especially in the shoots (< 9 mg/g, Table 3). In the short-term experiment, the Zn concentration in the shoots of plants grown in LDH-enriched sand media for 8 weeks was 6 (Zn-doped Mg-Fe-LDH-NO_3) and 5.5-fold (Zn-doped Mg-Fe-LDH-CO_3) higher compared to the control plants, while the concentration of Zn in the roots was similar in the treated and non-treated plants. These results indicate an effective translocation of Zn from the growth media to the shoots over 8 weeks.

When the growing time was increased to 11 weeks, the differences in the Zn concentration between treated and non-treated plants also increased (Table 3, Figure 5). The Zn concentration in the shoots of plants treated with Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3 was 9.5- and 7.9-fold higher, respectively, than the Zn concentration in the shoots of control plants. The corresponding difference for the roots was less pronounced (Table 3). The application of the nitrate form of the LDH significantly increased the Zn concentration in roots compared to the control plants, while no differences were found for the carbonate form. The same tendency of better efficiency of the nitrate LDH was observed in terms of the Zn accumulation in plants (Figure 5). A similar effectiveness at providing Zn to plants was observed in the short-term experiment after 8 weeks (Table 3 and Fig. 5).

Notably, the Zn concentration in the LDH-treated plants after 8 weeks was approximately 35 µg/g, and this value increased to 80 µg/g after 11 weeks, showing longer term release of Zn from the LDHs (Figure 5).

The results obtained in the long-term experiment after plants were grown in the LDH-enriched media for 28 weeks showed lower Zn concentration and Zn content in the treated plants than in the two previous experiments. However, a positive effect of Zn-doped Mg-Fe-LDH-NO_3 increasing the Zn concentration in shoots was observed compared to the control plants or to plants that had only received ammonium. The application of ammonium did not significantly enhance the effect of LDH. (Table 3 and Figure 5).

3.3.2. Dry matter production

The application of the Zn-doped Mg-Fe-LDHs did not affect the root DW of plants in any of the experiments. No differences were observed in the DW of shoots in the short-duration experiment after 8 weeks. However, a slightly higher shoot DW was recorded in Zn-doped Mg-Fe-LDH-treated plants (12% and 17% higher than the controls for Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3, respectively). In the longest experiment lasting 28 weeks, the application of ammonium alone and in combination with the Zn-doped Mg-Fe-LDHs increased the shoot DW of the plants (increases of 30% for Zn-doped Mg-Fe-LDHs combined with ammonium and 26% for ammonium application alone compared to control, Table 3). The shoot DW was not affected when treated only with the Zn-doped Mg-Fe-LDHs.

3.3.3. Effect of Zn-doped Mg-Fe-LDH fertilization on the uptake of other nutrients

Fertilization with the Zn-doped Mg-Fe-LDHs did not affect the macronutrient concentration in plants in the short- (8 weeks) or medium-duration (11 weeks) experiments (Supporting Information Table S2). In the long-duration experiment (28 weeks), significant differences were observed in the K and P concentrations, but not in the correspondent contents (g/plant).

The concentrations of Fe and Mn in the shoots of the non-treated control plants in the short-duration experiment were approximately 2 and 1.5 times higher than in the Zn-treated plants, respectively (Table 4). However, this effect was not observed in the medium-duration experiment, where no differences in these nutrients were detected between the Zn-doped Mg-Fe-LDH-treated and non-treated control plants. In the long-duration experiment, the Mn concentration in the roots of plants treated with Zn-doped Mg-Fe-LDH was reduced by 42% compared with that in the non-treated control plants. No differences in the Mn concentration in the roots were observed between the other treatments and the control in the long-duration experiment

3.4.Effect of Zn-doped Mg-Fe-LDHs applied to soil

No differences in dry weight or Zn concentration in shoots were observed after 3 weeks (Table 5). However, an increase in the Zn concentration in shoots of the plants treated with the Zn-doped Mg-Fe-LDHs was found after 7 weeks (Table 5). The Zn-doped Mg-Fe-LDH-NO_3 was the LDH which increased the Zn concentration at a comparable level to the Zn-EDTA. The application of the Zn-EDTA resulted in a higher Zn in soil as compared to the others at the end of the experiment. Slight differences between treatments were observed for the Mn and Cu concentrations at the early harvest but similar Fe, Mn and Cu concentrations were found in all the plants at the end of the experiment.

4. DISCUSSION

4.1.Solubility and nutrient release patterns of Zn-doped Mg-Fe-LDHs

Our first hypothesis was partially confirmed by the results obtained for the solubility of the Zn-doped Mg-Fe-LDHs in different extractants. The LDHs can be solubilized mainly in acidic media and remain stable in a neutral water pH. While partial dissolution was expected after extraction with 2% citric acid,

301 the values obtained were very close to those obtained from the total extraction with HCl for all the
302 nutrients. We hypothesized that this extraction solution may simulate the acidification processes
303 occurring in the rhizosphere when organic acids are released. However, the extraction efficiency was
304 much higher than that observed in the nutrient release experiment with changing pH and time in
305 aqueous solution (Table 2 and Figure 2) and in the plant experiments (Table 3 and Figure 5). By
306 contrast, the Zn concentration extracted by the DTPA-TEA solution[37] represented 32% and 44% of the
307 total Zn concentration in Zn-doped Mg-Fe-LDH-NO_3 and the Zn-doped Mg-Fe-LDH-CO_3,
308 respectively. These values are close to the maximum values of Zn in solution obtained in the nutrient
309 release experiment at the lowest pH studied, 5.2 (see detailed explanation below). Extraction with this
310 solution is a widely documented method for soil extraction used to predict the fraction of metal
311 micronutrients available to plants in soils with high pH. In our experiments, this extraction solution
312 proved to be a better method to predict the solubility of Zn from the Zn-doped Mg-Fe-LDHs at
313 rhizospheric pH than the extraction with citric acid. A negligible quantity of Fe was extracted by
314 DTPA-TEA, suggesting that, although Fe is present, it is potentially not released to plants but remains
315 as Fe (hydr)oxide precipitates over the pH range studied.

316 The dissolution kinetics of the pyroaurite Zn-doped Mg-Fe-LDH-NO_3 at different pH values was first
317 evaluated in a previous work [39] under controlled conditions in the absence of carbon dioxide for up to
318 more than 4 h (250 min). The release kinetics of Zn^{2+} and Mg^{2+} was first order with respect to the metal
319 cations in the LDH, providing rate constants in the range of 0.16×10^{-3} to 10.1×10^{-3} min^{-1}, with the
320 highest rate constants observed at low pH. A more in-depth investigation into the pH-dependent
321 dissolution of Zn^{2+} and Mg^{2+} from both Zn-doped Mg-Fe-LDH-NO_3 and Zn-doped Mg-Fe-LDH-CO_3
322 was performed here in the nutrient-release experiment in aqueous solution over 28 days. Similar to the

much shorter timespan previously studied, a clear pH dependence of Zn^{2+} and Mg^{2+} dissolution was observed and fitted to first-order curves, which confirms our first and second hypotheses. The data obtained demonstrate that total dissolution of the divalent cations from the LDHs is not possible at the studied pH values. Importantly, the maximum values ("a" constant in Table 2) are similar to those determined by DTPA-TEA extraction (pH 7.3) (Table 1). Moreover, a kinetic model that forecasts the Zn concentration that will be available to the plant showed that maximum dissolution was achieved during the first week. Similar to that found by Imran et al.[39] for non-doped Zn LDHs, the type of interlayer anion did not affect much the overall release kinetics. The experiment developed in the present work examined conditions closer to those in the soil solution, since the access to carbon dioxide was not restricted, and the reaction period was longer. In both experiments, Fe^{3+} was not released over the pH range of the study demonstrating that Fe^{3+} remained bound in the partially dissolved LDH or as an Fe (hydr)oxide, and that the LDH structure could be completely destroyed under only highly acidic conditions, as also observed by Imran et al.[39]

Although both Zn^{2+} and Mg^{2+} release from LDH at acidic pH was described here, Mg release is likely less interesting from the point of view of agricultural fertilizer practices, since Mg is a major element in plants, and the cost of applying the quantities needed by crops would presumably be too high when sourced from LDHs. An even stronger doubt could be raised concerning use of LDHs to control nitrate release to plants, considering costs. Nevertheless, the application of LDHs as N fertilizers has been widely documented, showing the controlled release of nitrate from these materials depending on the pH,[25–27,40,41] in addition to the use of LDH as a nitrate buffer to control excess nitrate in soils.[42,43]

4.2.Effects of length of growth period, pot geometry, and N source on plant Zn uptake in experiments with sand as growth medium

Similar Zn concentrations in plants were obtained in both the short- and medium-duration experiments (Table 3). Because of the higher plant biomass (observed in the plant DW) achieved in the medium-duration experiment compared with that in the short-term one, the Zn accumulated in the plants was approximately 3-fold higher in the former (Figure 5), which supports the idea that the synchronization of Zn from Zn-doped Mg-Fe-LDHs is adequate for at least 11 weeks (the duration of the medium-duration experiment) and is either continuously available or slowly released to the plant. This result agrees with the second hypothesis that the Zn-doped Mg-Fe-LDHs can fertilize soil in a slow-release manner that is controlled by the activity of the rhizosphere. A higher root mass leads to higher root activity and likely greater Zn release from the LDHs.

In the medium-duration experiment (considering both the Zn concentration and content, Table 3 and Figure 5), Zn-doped Mg-Fe-LDH-NO_3 was approximately 20% more efficient at providing Zn to plants than Zn-doped Mg-Fe-LDH-CO_3. By contrast, no differences were observed in the Zn release to aqueous solution of both LDH forms. According to the kinetic models of nutrient release (Eq. 1) (Figures 3 and 4), similar dissolution rates (expressed as "b") of the nitrate and carbonate forms occurred at the most acidic pH (Table 2).

Only 10% of the total Zn from the LDHs was taken up by plants. According to the curves obtained for the nutrient release in solution (Figure 3, Table 2) and the fraction extractable with DTPA-TEA, another 10% would be susceptible to release and made available to the plants in experiments of longer duration. This maximum, however, was not achieved in our long-duration experiment, which we ascribe to the experimental design used in this last experiment (see discussion below).

From the overall results obtained in the experiments, the Zn plant uptake was in line with the Zn extracted by DTPA-TEA and the nutrient release at the lowest pH but not with the extraction with citric acid. Although this extraction method was suggested as a potential predictor of the Zn dissolved in the rhizosphere and thus the Zn uptake, the traditional DTPA-TEA method for soils resulted in a more reliable method to predict the Zn available to plants from the Zn-doped Mg-Fe-LDH fertilizers. According to these results, we can hypothesize that a larger Zn quantity is dissolved in the rhizosphere while only a smaller portion is taken up by the plant. These results illustrate the limitation of chemical extractants in predicting the Zn available to plants, highlighting the importance of using plant-soil experiments.

In addition to the Zn-doped Mg-Fe-LDH effect alone, a possible synergistic effect when applied with N as ammonium was explored in our long-duration experiment, since ammonium application can cause rhizosphere acidification by inducing proton release from roots.[44] Thus, ammonium uptake was hypothesized to stimulate the dissolution of Zn-doped Mg-Fe-LDHs. According to the results obtained in this experiment, a greater Zn release from Zn-doped Mg-Fe-LDH occurred in the treatment combining Zn-doped Mg-Fe-LDH and ammonium, leading to the highest Zn concentration in the roots. However, this enhanced effect was not observed in the shoots during the experimental period, as we had hypothesized. In addition to the enhanced effect of ammonium on Zn-doped Mg-Fe-LDH dissolution and the longer duration (28 weeks), the Zn-doped Mg-Fe-LDH placement in the last experiment was different compared to those of the other experiments. In the short- and medium-duration experiments, small pots were used, and the LDHs were mixed with a portion of sand that was equivalent to 31 and 30% of the total sand in the pot, respectively. In the long-duration experiment, a much smaller portion of sand was used to prepare the mixture with the LDHs (4% of the total sand in

387 the pot) and the fertilized sand was the bottom of the pot. The initial purpose of this experimental
388 design was, in addition to evaluating the Zn uptake, to visualize the possible accumulation in the roots
389 in the layer containing the fertilizers, which was, however, not clearly observed. In contrast, the roots
390 were homogenously distributed along the pot, and consequently, only a minor part of the total root
391 volume was in contact with the Zn-doped Mg-Fe-LDH placed as a thin layer in the pot (Figure 2),
392 which could explain both the lower Zn concentration and contents (Table 3 and Figure 5) detected in
393 the oldest plants compared to those in the short- and medium-duration experiments. According to
394 Zhang et al.,[45] lower Zn concentrations were found in plants cultivated in pots in which Zn fertilization
395 was applied in the bottom half. We therefore believe that the fertilizer placement in our long-duration
396 experiment was not adequate, since the fertilizer was situated far from the seed and probably did not
397 interact much with the rhizosphere due to the large pot size. Nevertheless, the results obtained indicate
398 that the Zn-doped Mg-Fe-LDHs worked even over the long-term period in restricted volumes, which is
399 closer to a realistic scenario for agronomic fertilization.

400 **4.3.Plant Zn uptake in soil experiment**

401 An effective Zn supply to plants was also observed following application of Zn-doped Mg-Fe-LDH-
402 NO_3 to a calcareous soil, which confirms their potential as Zn fertilizers. Considering the complexity of
403 factors affecting the release of Zn from the Zn-doped Mg-Fe-LDHs and the subsequent uptake by
404 plants (see discussion above), together with the fact that soil is much more reactive than sand, a more
405 prominent effect may be expected in experiments with longer duration.

406 **4.4.Effect of Zn-doped Mg-Fe-LDH fertilization on other nutrients**

The concentrations of macronutrients and other micronutrients than Zn were adequate in all the plants. However, higher Fe and Mn concentrations in the shoots of control Zn-deficient plants were observed in the short-duration experiment (Table 4), although the nutrient solution provided by irrigation in the experiments did not contain Zn or Fe. As previously indicated, the initial hypothesis was to explore the possibility of Fe fertilization using LDHs, besides using Zn. Although the studied Zn-doped Mg-Fe-LDHs contained substantial quantities of Fe (see Table 1), this Fe was not available to the plants in the conditions used. The LDH characterization results indicate that, although the total Fe in the LDHs was approximately 25% by weight, the Fe available to plants (based on DTPA-TEA extraction) was several orders of magnitude lower. Moreover, the experiment studying the nutrient release over time at different pH values confirmed the structural function of Fe^{3+} in the metal hydroxide layer of the LDHs and also that it was not possible to release Fe^{3+} at agronomically relevant pH values.

The higher Fe and Mn in the control plants (Table 4) may have been associated with different factors. Based on physiological mechanisms of micronutrient acquisition by plants, the Zn deficiency has been shown to increase the Fe concentration in the shoots possibly due to mechanisms involving acidification of the rhizosphere and the release of reductants and phytosiderophores (PS).[46] Root exudates from Zn-deficient plants have been shown to mobilize more Fe from Fe (hydr)oxides than the exudates from Zn-adequate plants, [47] and more Fe has been shown to mobilize from roots to shoots in Zn-deficient cultivars.[48–50] To the best of our knowledge, no studies have been found in the literature on the effect of Zn deficiency on Mn uptake. However, considering that Mn uptake shares the same acquisition tentative mechanisms as for Zn and Fe,[51] a similar behavior may be expected. Based on the chemistry of these metals in soils, their precipitation as metal (hydr)oxides is expected under alkaline conditions.[52] Zn-doped Mg-Fe-LDH dissolution increases the surrounding pH, as observed during the

setup of the nutrient-release assays at different pH values and times, which was the reason for adding high buffer concentrations. Thus, alkalinization of the growth media due to the dissolution of Zn from LDH could induce the precipitation of other metals such as Fe and Mn, reducing their availability to plants.

This secondary effect on Fe and Mn nutrition was not observed in the medium- or long-duration experiments, suggesting that if Fe and Mn are retained due to precipitation, they can be subsequently released over time and become available to the plant. In future experiments, Fe should be provided within the nutrient solution to avoid this possible interaction.

No reduction of the Fe and Mn concentration in control plants following application of Zn-doped Mg-Fe-LDHs was observed in the soil experiment (Table 5). The likely reflects that soil-borne micronutrients were supplementing those added with the LDHs. In fact, the concentrations of Mn and Cu were slightly increased by the application of the Zn-doped Mg-Fe-LDH-CO_3 and the ZnEDTA as compared to control plants (Table 5).

Based on the results obtained in this work, a mechanistic model of Zn dissolution from the Zn-doped Mg-Fe-LDHs and their subsequent root uptake is proposed in Figure 6. At the particle surface, Zn dissolution can be described in two steps: the rapid formation of reactive sites on the surface through the protonation of hydroxyl groups (Figure 6-I) and the detachment of cations from the particle surface (Figure 6-II).[53] The first step would be promoted by the release of H^+, OAHs, and PS to the rhizosphere under Zn-deficient conditions (Figure 6-I), followed by Zn detachment from the layer (Fig. 6-II) and subsequent root uptake as Zn^{2+} or PS-Zn (Fig. 6-III). In addition, we hypothesize that the resulting gap in the layer could be occupied by other Zn(II) ions or similar divalent metals adsorbed in the soil or

450 growth medium (Fig. 6-IV), such as Mn^{2+} or Fe^{2+}, which could be subsequently released to the plant by
451 the same mechanisms described above.

452 To conclude, an effective Zn release from LDHs has been demonstrated in the present work at acidic
453 pH values, similar to those occurring in the rhizosphere. Barley plants growing in Zn-doped Mg-Fe-
454 LDH quartz sand media increased their shoot and root Zn concentrations in three experiments of short,
455 medium and long durations. The pH-dependent dissolution of the Zn-doped Mg-Fe-LDHs and the
456 effective Zn uptake by barley plants in the long-term sand experiment as well as in the soil experiment
457 supports that the release of Zn is rhizosphere. This work provides proof-of-concept that Zn-doped Mg-
458 Fe-LDHs may be used as Zn fertilizers. The Zn in zinc-doped LDHs may maintain its plant-availability
459 for a longer period making Zn-doped Mg-Fe-LDHs making Zn-doped Mg-Fe-LDHs a potentially
460 effective and alternative to traditional inorganic Zn sources.

461 **ACKNOWLEDGMENTS AND FOUNDING**

462 This work was supported by the Danish Council for Independent Research, Technology and Production
463 Sciences (Project Number 0602-02111B FTP) and by the Horizon 2020-Marie Skłodowska-Curie
464 actions Intra-European fellowship (IF) "MicroFert" project of SLR. The authors are grateful to Prof.
465 José Maria García-Mina for his scientific advice.

466 **Supporting Information Available:** Spectroscopic analysis of the synthetized Zn-doped Mg-Fe-LDHs
467 and formula determination, total nutrients applied by the nutrient solution and macronutrient
468 concentrations in plants grown in sand. This material is available free of charge via the Internet at
469 http://pubs.acs.org

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Figure Captions

Figure 1. Structure of Layered Double Hydroxide (LDH).

Figure 2. Design and main characteristics of the experimental setup in the short-, medium- and long-duration plant experiments. Zn-doped Mg-Fe-LDH-NO_3 (Zn-LDH-NO_3), Zn-doped Mg-Fe-LDH-CO_3 (Zn-LDH-CO_3), ammonium (Amm).

Figure 3. Zn^{2+} release over time from Zn-doped Mg-Fe-LDH-NO_3 (Zn-LDH-NO_3, left) and Zn-doped Mg-Fe-LDH-CO_3 (Zn-LDH-CO_3, right) incubated at different pH levels for up to 28 days. Points are the means, and bars are the SE (N=2). Dashed lines indicate the total Zn concentration in the LDHs (see Table 1).

Figure 4. Mg^{2+} release over time from Zn-doped Mg-Fe-LDH-NO_3 ((Zn-LDH-NO_3, left) and Zn-doped Mg-Fe-LDH-CO_3 (Zn-LDH-CO_3, right) incubated at different pH levels for up to 28 days. Points are the means, and bars are the SE (N=2). Dashed lines indicate the total Mg concentration in the LDHs (see Table 1).

Figure 5. Total Zn contents (µg/plant) in the shoots and roots of barley plants at the end of the short- (8 weeks), medium- (11 weeks) and long-duration (28 weeks) experiments. Error bars are the standard errors (N=5). Different letters indicate significant differences between treatments for the shoots or roots in each experiment according to the Tukey post hoc test ($P<0.05$). . Zn-doped Mg-Fe-LDH-NO_3 (Zn-LDH-NO_3), Zn-doped Mg-Fe-LDH-CO_3 (Zn-LDH-CO_3), ammonium (Amm).

Figure 6. Proposed mechanisms for release of Zn^{2+} and Mg^{2+} and subsequent root uptake. See explanation in the text.

622 **Table 1.** Nutrient extracted (in mg/g LDH) from Zn-doped Mg-Fe-LDHs using HCl (35%), citric acid

623 (2%), DTPA (50 mM), and water. Values are means±SD (N=3).

	Zn-doped Mg-Fe-LDH-NO_3			**Zn-doped Mg-Fe-LDH-CO_3**		
	Mg (mg/g)	Fe (mg/g)	Zn (mg/g)	Mg (mg/g)	Fe (mg/g)	Zn (mg/g)
HCl (Total)	187 ± 11	249 ± 12	44.6 ± 2.8	206 ± 9	269 ± 11	45.6 ± 1.5
Citric acid	181 ± 03	24.7 ± 2	43.0 ± 0.6	194 ± 5	260 ± 06	42.8 ± 1.2
DTPA	62.4 ± 0.7	0.0206 ± 0.000	14.5 ± 0.0	72 ± 2	0.0214 ± 0.001	20.3 ± 0.4
Water	<LD	<LD	<LD	<LD	<LD	<LD

624 <LD indicates values under the limit of detection.

625

Table 2. Fitting curve parameters for Zn^{2+} and Mg^{2+} release from the Zn-doped Mg-Fe-LDHs at different pH values (N=2).

Zn-doped Mg-Fe-LDH	Cation	pH	a (mg M^{2+}/g LDH)	b (day^{-1})
Zn-doped Mg-Fe-LDH-NO_3	Zn^{2+}	5.2	20.3 (±0.8)***	0.552 (±0.090)**
		5.5	18.4 (±1.0)***	0.534 (±0.120)*
		6	15.8 (±0.6)***	0.389 (±0.058)**
		7.1	3.69 (±0.2)***	0.324 (±0.064)**
		8.1	0.0851 (±0.0156)**	0.249 (±0.143)
	Mg^{2+}	5.2	139 (±8)***	1.48 (±0.59)
		5.5	129 (±7)***	1.45 (±0.46)*
		6	136 (±4)***	1.11 (±0.19)**
		7.1	129 (±4)***	1.10 (±0.16)**
		8.1	71 (±5)***	0.648 (±0.213)*
Zn-doped Mg-Fe-LDH-CO_3	Zn^{2+}	5.2	18.8 (±0.8)***	0.546 (±0.107)**
		5.5	17.9 (±0.7)***	0.784 (±0.147)**
		6	14.6 (±0.5)***	0.502 (±0.0706)**
		7.1	3.08 (±0.22)***	0.439 (±0.124)*
		8.1	0.0954 (±0.0119)**	0.127 (±0.041)*
	Mg^{2+}	5.2	128 (±3)***	2.79 (±0.88)*
		5.5	131 (±2)***	2.48 (±0.48)**
		6	133 (±1)***	1.11 (±0.07)***
		7.1	107 (±6)***	1.15 (±0.33)*
		8.1	54.7 (±7.1)**	0.861 (±0.551)

(Standard error shown in parentheses)

P-values obtained for the model are indicated by asterisks: *P<0.05, **P<0.01, ***P<0.001.

Table 3. Dry weight (DW) and Zn concentration in shoots and roots of barley plants at the end of the short- (8 weeks), medium- (11 weeks) and long-duration (28 weeks) experiments. Different letters in each column for each parameter indicate significant differences between treatments according to the Tukey post hoc test ($P<0.05$); ns: not significant. Values are means for five replicates.

Short-duration experiment (8 weeks)

	Shoot		Root	
Treatments	DW (g/plant)	Zn concentration (µg/g DW)	DW (g/plant)	Zn concentration (µg/g DW)
Zn-doped Mg-Fe-LDH-NO_3	0.297 ns	51.1 a	0.166 ns	109 ns
Zn-doped Mg-Fe-LDH-CO_3	0.288	47.1 a	0.172	109
Control	0.279	8.50 b	0.155	91.0

Medium-duration experiment (11 weeks)

	Shoot		Root	
Treatments	DW (g/plant)	Zn concentration (µg/g DW)	DW (g/plant)	Zn concentration (µg/g DW)
Zn-doped Mg-Fe-LDH-NO_3	0.841 a	59.2 a	0.386 ns	80.6 a
Zn-doped Mg-Fe-LDH-CO_3	0.876 a	49.2 b	0.376	55.0 ab
Control	0.745 b	6.21 c	0.322	26.2 b

Long-duration experiment (28 weeks)

	Shoot		Root	
Treatments	DW (g/plant)	Zn concentration (µg/g DW)	DW (g/plant)	Zn concentration (µg/g DW)
Zn-doped Mg-Fe-LDH-NO_3 + Ammonium	1.14 a	12.5 a	1.33 ns	21.0 a
Zn-doped Mg-Fe-LDH-NO_3	1.04 ab	12.8 a	1.45	18.6 ab
Ammonium	1.11 a	7.06 b	1.60	14.5 ab
Control	0.880 b	7.00 b	1.06	13.9 b

635 **Table 4.** Fe, Mn, and Cu concentration in the shoots and roots of barley plants at the end of the short-
636 (8 weeks), medium- (11 weeks) and long-duration (28 weeks) experiments. Different letters in each
637 column for each parameter indicate significant differences between treatments according to the Tukey
638 post hoc test ($P<0.05$); ns: not significant. Values are means for five replicates.

Short-duration experiment (8 weeks)

	Shoot (µg/g DW)			Root (µg/g DW)		
Treatments	Fe	Mn	Cu	Fe	Mn	Cu
Zn-doped Mg-Fe-LDH-NO_3	42.9 b	41.1 b	9.9 ns	202 ns	31.5 b	29.0 a
Zn-doped Mg-Fe-LDH-CO_3	40.7 b	38.3 b	9.29	191	46.4 ab	29.6 a
Control	87.5 a	59.82 a	9.36	267	59.7 a	41.9 b

Medium-duration experiment (11 weeks)

	Shoot (µg/g DW)			Root (µg/g DW)		
Treatments	Fe	Mn	Cu	Fe	Mn	Cu
Zn-doped Mg-Fe-LDH-NO_3	40.4 ns	45.4 ns	11.3 ns	232 ns	33.1 ns	32.8 ns
Zn-doped Mg-Fe-LDH-CO_3	42.2	53.7	10.0	184	54.9	32.0
Control	45.4	44.7	9.51	264	38.9	37.1

Long-duration experiment (28 weeks)

	Shoot (µg/g DW)			Root (µg/g DW)		
Treatments	Fe	Mn	Cu	Fe	Mn	Cu
Zn-doped Mg-Fe-LDH-NO_3 + Ammonium	21.6 ns	82.4 ns	4.44 ns	910 ns	31.9 ab	11.2 ns
Zn-doped Mg-Fe-LDH-NO_3	18.1	90.3	4.29	795	26.2 b	9.2
Ammonium	17.8	128	5.57	839	38.9 ab	10.0
Control	20.9	152	4.81	773	45.6 a	10.6

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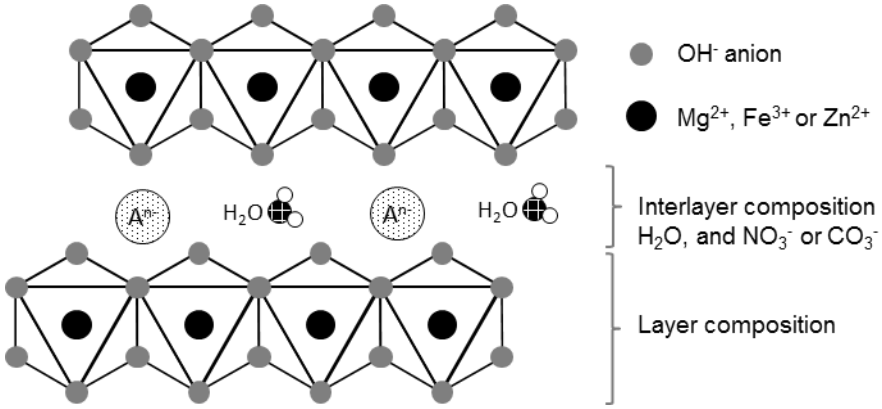
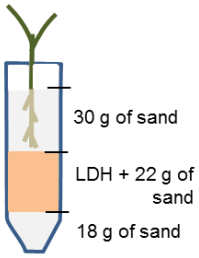
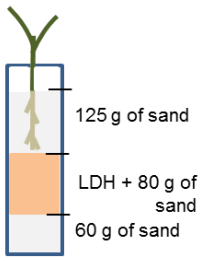
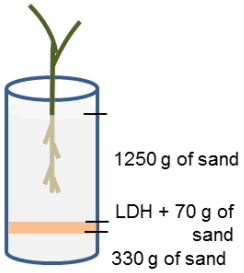
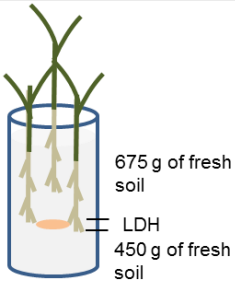
Table 5. Dry weigh (DW) and Zn, Fe, Mn and Cu concentration in the shoots of barley plants growing in a calcareous soil. Different letters in each line indicate significant differences between treatments according to the Tukey post hoc test ($P<0.05$); ns: not significant. Values are means for five replicates.

		Treatments			
		Control	Zn-doped Mg-Fe-LDH-NO_3	Zn-doped Mg-Fe-LDH-CO_3	ZnEDTA
3 weeks	DW (g/plant)	0.146 ns	0.151	0.160	0.167
	Shoot concentration (µg/g DW)				
	Zn	35.9 ns	36.0	38.6	41.1
	Fe	47.6 ns	47.2	51.1	52.3
	Mn	4.56 b	4.88 ab	5.10 a	5.34 a
	Cu	5.84 c	6.14 bc	6.82 ab	7.04 a
7 weeks	DW (g/plant)	0.929 ab	0.889 b	1.02 ab	1.19 a
	Shoot concentration (µg/g DW)				
	Zn	31.5 c	38.5 ab	33.2 bc	39.9 a
	Fe	35.4 ns	39.9	35.8	36.6
	Mn	3.64 ns	3.86	3.78	3.86
	Cu	4.92 ns	5.52	5.08	5.20
	Zn soil concentration (µg/g DW)	1.40 b	1.25 b	1.38 b	1.89 a

Figure 1


OH- anion
Mg2+, Fe3+ or Zn2+
An-
H2O
An-
H2O
Interlayer composition
H2O, and NO3- or CO3-
Layer composition

651 **Figure 2**

Experiment (duration)	**Short** (8 weeks)	**Medium** (11 weeks)	**Long** (28 weeks)	**Soil exp** (7 weeks)
Scheme Pots				
Zn-LDH dose/pot	10 mg	10 mg	10 mg	33 mg
Treatments	Zn-LDH-NO_3 Zn-LDH-CO_3 Control –Fe-Zn	Zn-LDH-NO_3 Zn-LDH-CO_3 Control –Fe-Zn	Zn-LDH-NO_3 Zn-LDH-NO_3 + Amm Control –Fe-Zn Control –Fe-Zn+ Amm	Zn-LDH-NO_3 Zn-LDH-CO_3 ZnEDTA Control –Zn
Pot volume	50 ml	160 ml	1100 ml	1800 ml
Total growing media/pot	70 g	265 g	1650 g	1125 g

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654 **Figure 3**

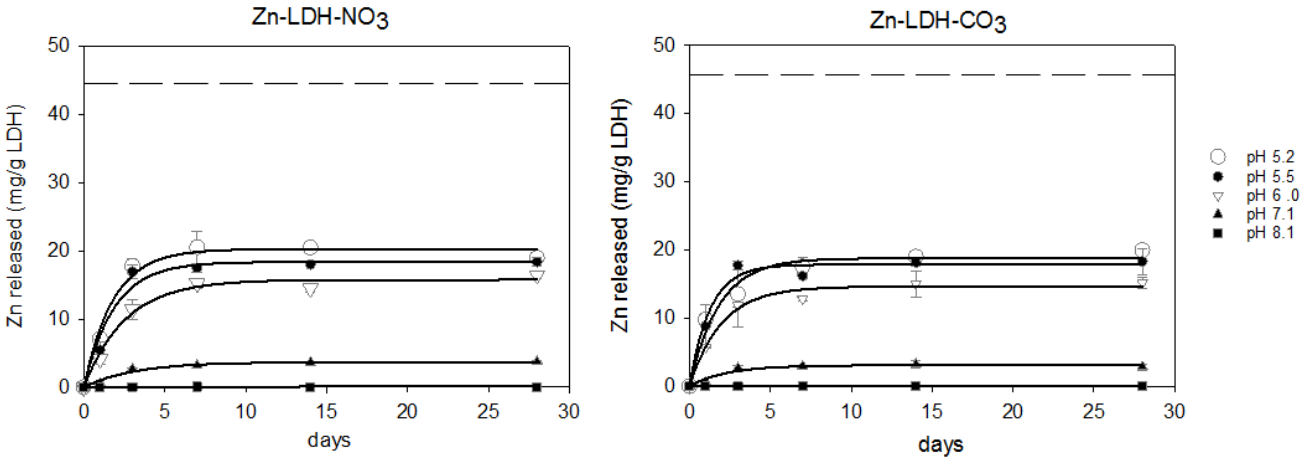

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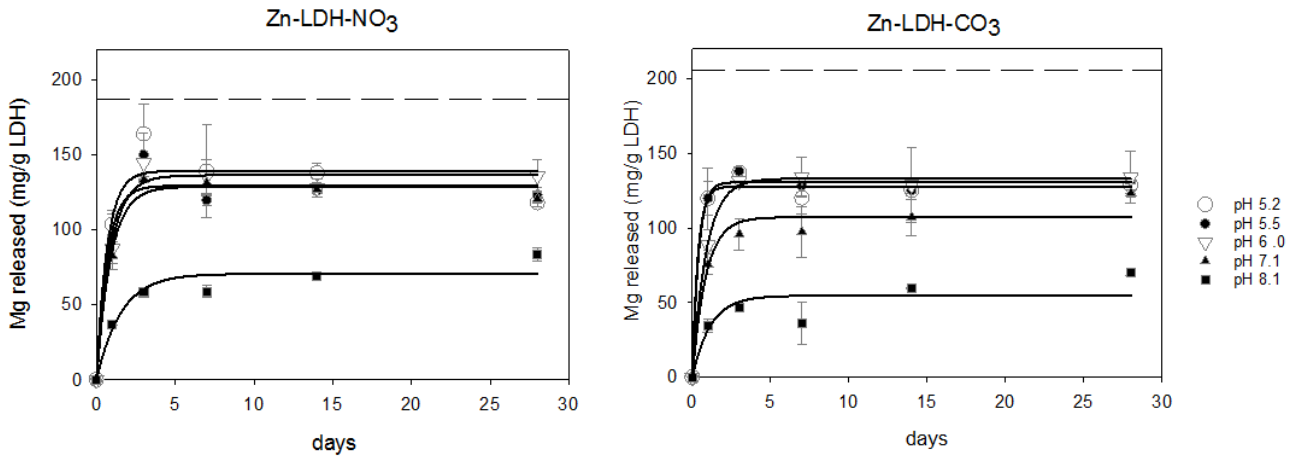
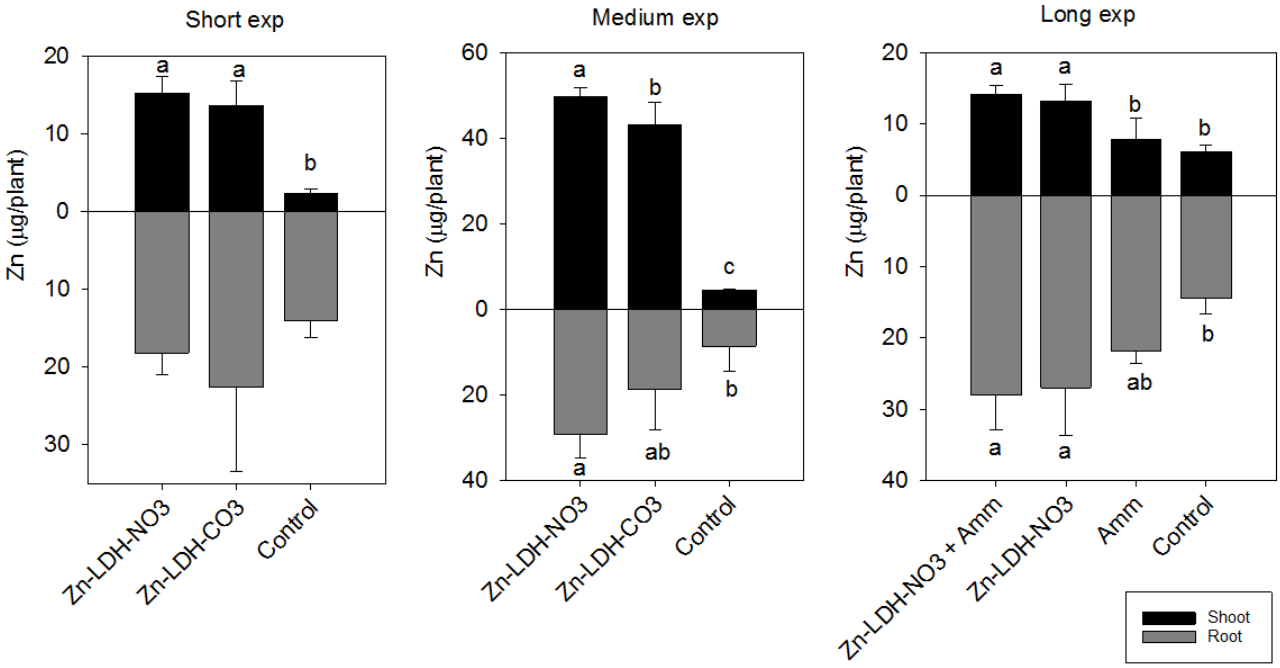
Figure 4


Zn-LDH-NO3
Zn-LDH-CO3
Mg released (mg/g LDH)
days
0
50
100
150
200
0
5
10
15
20
25
30
pH 5.2
pH 5.5
pH 6 .0
pH 7.1
pH 8.1

660 **Figure 5**



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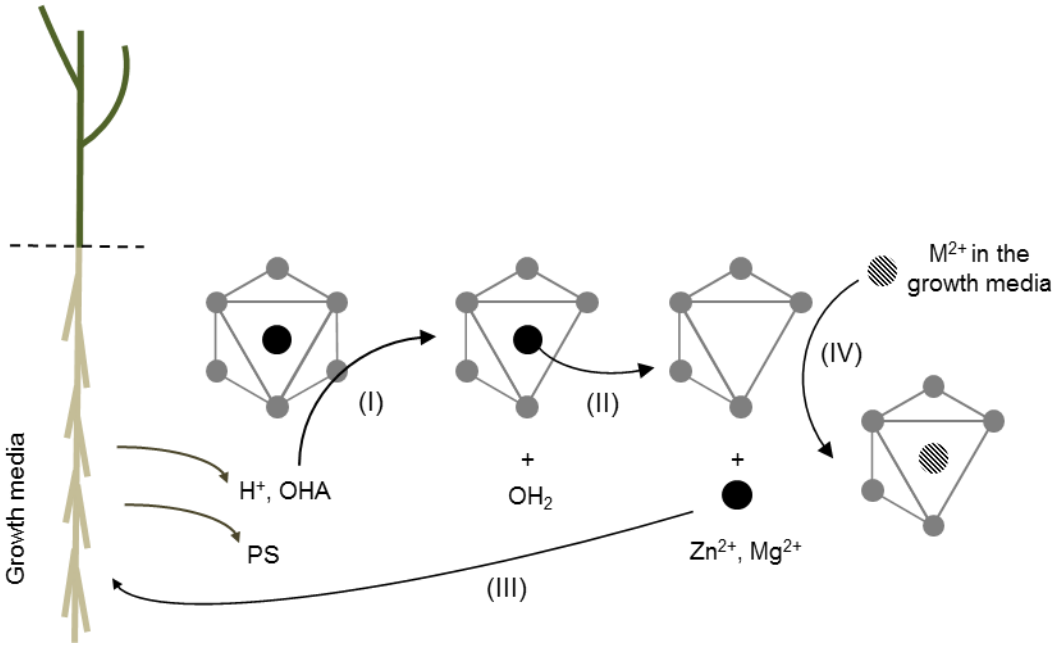
663 **Figure 6**



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